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Managing Editor
World Journal of Gastroenterology

Re: BPG manuscript Number 53988

Dear Editor:

It is my great pleasure to send in the revised manuscript entitled "*Type I and Type II Helicobacter pylori Infection Status in Stepwise Chronic Gastric Diseases and Their Impact on Gastrin and Pepsinogen Level In a High Gastric Cancer Prevalent Area*" to World Journal of Gastroenterology for evaluation.

The manuscript has been revised based according to reviewers' suggestions, a point-by-point reply to the reviewer's question is also provided in the next 7 pages. We hope this revised version will satisfy both editors and reviewers. In addition, the required accompanying documents are also uploaded via the F6Publishing system for your information.

(1) 53988-Manuscript File
(2) 53988-Answering Reviewers
(3) 53988-Audio Core Tip
(4) 53988-Conflict-of-Interest Disclosure Form
(5) 53988-Copyright License Agreement
(6) 53988-Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)
(7) 53988-Non-Native Speakers of English Editing Certificate
(8) 53988-Signed Informed Consent Form(s) or Document(s)
(9) 53988-STROBE checklist
(10) 53988-Supplementary figures
(11) 53988-Institutional Review Board Approval Form or Document
(12) 53988-Biostatistics Review Certificate

Thank you very much for your time and attention, and hope the manuscript can be favorably considered!

With Best Regards,
Sincerely yours,

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Reviewer 1 **Date: 2020-01-04 13:15**

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments To Authors: MS ID: 53988 TITLE Type I and Type II Helicobacter pylori Infection Status in Stepwise Chronic Gastric Diseases and Their Impact on Gastrin and Pepsinogen Level In a High Gastric Cancer Prevalent Area

It is a potentially interesting study indicating that Type I H. pylori infection account for 84.2% of all gastric cancer, and has a significant impact on G-17 and pepsinogen levels. However, authors indicated that Type II H. pylori infection account for only 15.8% of all gastric cancer, and had(s) a minimum impact on G-17 and pepsinogens. Based on these findings, the one possible reason why Type II H. pylori infection does not have a strong virulence is that it has less serological impact compared to Type I H. pylori strains. However, there were several issues that concern me.

Major points

1) Author should refer to the results of previous reports concerning the risk of Type 2 H. pylori infection on gastric cancer development. And if there were (if there were) previous reports, their data should be discussed.

Author reply to reviewer:

Authors have searched extensively on the PubMed and could not found related reports on the impact of type II *H. pylori* infection on the occurrence of gastric cancer. However, there are huge numbers of studies on the virulent *H. pylori* that carry cagPAI, CagA, VacA and their impacts on gastric cancer occurrence. This is indeed an interesting topic which deserves future investigation.

2) Authors should refer to the histological type of gastric cancer both in Type1 and Type 2 H. pylori strains.

Author reply to reviewer:

The histological type of all 43 gastric cancer patients was intestinal type of gastric cancer. We have put this information in the results part in lines 226-228.

3) I consider that serological findings of CAG in H. pylori negative, Type1 and Type 2 positive subjects are especially important. It seems to be that PGII in Hp (-) subjects (N=51, 8.7±4.3) is significantly lower ($p<0.01$) than that of Type 2 Hp (+) subjects (N=13, 15.4 ±9.6) using student t test. Please re-evaluate the statistics especially in Table 5.

Author reply to reviewer:

Authors have recalculated PGII values of type II *H. pylori*-positive and *H.pylori*-negative patients in CAG group with student "*t"* test and modified the results in Table 5. Serum PG II values in type II *H. pylori*-positive (N=13, 15.4±9.6) patients were significantly higher than those of *H. pylori*-negative patients (N=51, 8.7±4.3) (T= -2.554, P=0.017), but results from other groups did not reach statistical significance at this stage. Authors are grateful to reviewer to point this out.

4) Authors should refer to the mechanisms of being a weak virulent factors of Type 2 H. pylori infection. It seems to me that the development rate of CAG in Type 2 H. pylori strains is low (Type 1: 79.7% vs Type 2: 20.3%), which is the main reason of low virulent factors. Thus, I speculate that it is understandable that type 2 strain have impact on PGII and PGI/II ratio. Please refer to the pathophysiological mechanisms of the difference of the virulent factors.

Author reply to reviewer:

Compared with *H. pylori*-negative patients, serum PGII level in *H. pylori* infected patients was higher and PG I/PG II ratio was lower, and this effect was mostly from type I strain infection (in Table 4). Also in CAG patients, PG II levels in type I *H. pylori*- and type II *H. pylori*-infected patients were significantly higher, and PG I/PG II ratio was lower than those in *H. pylori*-negative patients ($P<0.05$), so type I and type II *H. pylori* strain all have an impact on PG I/PG II ratio, but this effect was weaker from type II *H. pylori*. The mechanism is probably due to the inflammation caused by the virulent factors, such as cagPAI, CagA, VacA and others, this has been discussed in Discussion in the first two paragraphs and in lines 388-391.

5) H. pylori negative gastric cancer is within 1% in Japan. Although authors stated in Discussion that "The current results are in line with these results, and indicate an important role of type I H. pylori in the development of upper gastrointestinal diseases and gastric cancer", 11.6 % seems to be relatively higher than that of Japanese report. I consider that H. pylori negative cases defined by this author are negative for serology and UBT, however, past infection cases or spontaneously disappeared cases are inevitably included. Authors should define it as at least subjects excluding PG positive cases. Data may be improved if definition of H. pylori more precisely.

Author reply to reviewer:

We thank the reviewer to point this out, the reason for this discrepancy is currently unknown, and we thought this could be a geographic or life-style variation, but is worth further study. To avoid false positive or negative and increase accuracy, authors used both ^{13}C-BUT and serological *H. pylori* antibody positive tests as a diagnostic criterion to define *H. pylori* positive, patients who were either ^{13}C-UBT or serological *H. pylori* antibody positive, but not both, were not enrolled in this study, this was discussed in lines 154-157. In addition, patients were questioned for past infection and treatment history before enrollment.

Reviewer 2 Date: 2020-01-14 07:04

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments To Authors:

This manuscript studied H. pyroli infection and serum biomarkers for gastric cancer in the patients of various gastric diseases. Though enrolled cases were enough to support the conclusion, the results were not unique except the infection status of H. pyroli in Henan area. I recommend some suggestions to highlight the results.

Major

1. Could you suggest diagnostic values (or ROC) for G17 and pepsinogen to DDx NAG, NAGE, CAG, PU and GC.

Author reply to reviewer:

As advised by reviewer, authors have recalculated data and made receiver operating curves (ROC) for CAG, PU and GC diagnosed by G17 and pepsinogens, this was shown in Figure 2 in results and discussed properly in discussion part.

2. Change Table 2 and 4 into Figure

Author reply to reviewer:

Authors have considered suggestions by reviewer, and believe that Tables 2 and 4 are more informative and contain percentages derived from the data, therefore would like to retain the original table 2 and 4 in the draft, but also translated the table 2 and 4 into supplementary S-Figures 1 and 2 and submitted as supplementary figure with blot data for reader and reviewer's information if this is in line with journal policy.

3. Table 5; Describe total level of G17, PG I, PG II and PGI /II in each group

Author reply to reviewer:

This was added in each group in Table 5 as advised by reviewer.

Minor

1. Abst line 72; expect in GC patients > except in GC patients

Author reply to reviewer:

The author has modified it in the manuscript, thanks reviewer to mention this.

2. Line 308: diffuse type types of gastric cancer are > correct type types

Author reply to reviewer:

This has been changed in text, and authors are grateful to reviewer to correct this.

3. Fig 1; Complete the sentence at 462 patients were

Author reply to reviewer:

Authors are grateful to reviewer to mention this, and it has been changed in Figure 1.

1) Describe Mean age ±SD in Hp +, Type 1 Hp+, Type II Hp+ and Hp- group

Author reply to reviewer:

Authors have described mean age ±SD in Hp +, Type I Hp+, Type II Hp+ and Hp- groups.

2) There were no clinically significant correlation Patients characteristics and H. pylori infection. Change the title into Patient Clinical Data and H. pylori Infection Status (also in Text).

Author reply to reviewer:

Author have changed it into "Patient Clinical Data and *H. pylori* Infection Status" in both title and text.

Review 3 Date: 2020-01-26 22:08

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments To Authors:

Yuan et al. studied the H. pylori status in a high-gastric cancer prevalence area. They focus on differ H. pylori types and evaluate them within the scope of histology and PGI, PGII and G17. This topic is interesting and there is an ongoing change in prevalence. Furthermore, there is a(n) ongoing debate regarding the value of PG I, PG II and G17 in prediction of preneoplastic conditions and potential risk of gastric cancer.

- Introduction: rather short and the last part of the introduction included some redundant result section.

Author reply to reviewer:

Authors tried to keep the manuscript concise and up-to-the-point, the redundant part in the introduction was removed.

- Please explain what is the benefit to create a NAGE group, usually it is part of the NAG unless atrophy or ulcer is present.

Author reply to reviewer:

The degree of gastric inflammation in NAGE group was higher than that in NAG group, but did not meet the criteria for atrophy or ulcer, we intended to take a look of those patients with erosions in the mucosa and compared if they make any difference in disease progression, this is why an additional group was created, but the results of NAGE group was almost same as the NAG group.

- Ethics: Research protocol number is not included. I have my strong concerns regarding the verbal consent which is inappropriate for the high-quality research, unless the all the analysis is a standard of care and the study had an exempt from the ethical committee, however, in this case it is a wrong statement in the paper.

Author reply to reviewer:

All subjects signed informed consent and gave verbal consent before being examined and enrolled, we have made changes in the text, and protocol number was also added in the text (line 150-151).

- Information to the H. pylori blot is not provided. Recently there is a report in WJG (World J Gastroenterol. 2017 Jul 14;23(26):4712-4723) showing that cagA antibody production is dependent on vacA genotype. In this regard, the authors may need to discuss the data in view of the published results.

Author reply to reviewer:

We have put one representative blot picture in the supplementary figure for information.

The relation of CagA antibody production and *vacA* genotype is indeed an interesting topic as presented by Dr. Link A., *et al* from Prof. Malfertheiner's group in 2017 WJG paper, the results indicated that 30.3% of *H. pylori* infected patients were positive anti-CagA-IgG serology; the immune response to CagA may be in part triggered by the effect of VacA on the gastric mucosa, but authors also indicted that anti-CagA-seropositivity varies between different regions with highest prevalence in Asian countries and lowest in Europe. In our results, CagA positivity is 70.1%, VacA positivity is 61.9% in Hp infected patients (Table 3), although we did not examine their relationship at this work, the results appear varied greatly between the two geographic areas.

Given the amount of work load in the current manuscript and different aims, this work is not designed to or capable to differentiate CagA,VacA relationship at this stage, authors do agree with reviewer that this is indeed a interesting and important question. We will take a look in next project to see if the new informaiton may apply to this geographic region. Authors are very grateful to the reviewer to point this question out!

- In results, probably it is appropriate to mention the PU before CAG in results as CAG is already preneoplastic condition, while peptic ulcer not.

Author reply to reviewer:

The sequence of PU, CAG in Results section has been changed in both text and tables as advised.

- CAG is a mix of the atrophy types: corpus or antrum atrophy. The paper would benefit from more detailed data if possible.

Author reply to reviewer:

In this study, chronic atrophic gastritis included atrophy of antrum and corpus, 48 patients were antrum atrophy and 29 were corpus atrophic gastritis; although there were variations in degree and area, they were categorized in the same CAG group. This is indeed a concern as extensive atrophy and spotted, localized atrophy would behave very differently in disease progress. Our results indicated 83.1% of CAG patents are infected by *H. pylori* and type I Hp account for 79.7% of them, indicated a major role in etiology, antrum atrophy is the predominant form, we have put this in the results part in lines 226-228 as advised by reviewer.

- How many of the H. pylori negative subjects had negative serology for cagA. It is for instance known that H. pylori-antibody disappear after eradication, while cagA-IgG antibody remain positive in a cohort of patients (World J Gastroenterol. 2017 Jul 14;23(26):4712-4723).

Author reply to reviewer:

The status of *H. pylori* infection were confirmed by both ^{13}C-UBT and serological *H. pylori* antibody test, patients were considered *H. pylori* negative when both tests were negative, their CagA, VacA, UreA, and UreB antibodies were all negative in total 121 *H. pylori*-negative patients. When patients were either ^{13}C-UBT or serological *H. pylori* antibody positive, but not both, they were not enrolled to avoid false-positive or -negative, the information was listed in lines 154-157. But it is indeed a great question to study further of CagA antibody positivity after bacteria eradication.

- How many PU subjects were on PPI? This would explain increased G17 values.

Author reply to reviewer:

All subjects enrolled in this cohort did not have a history of taking proton pump inhibitors, bismuth salts, H_2-receptor blockers or other medications that might affect test results over the past two weeks (lines 135-142). There might be other reasons for increased G-17 level as discussed in Discussion section, lines 388-391.

- I am quite surprised of the very high PG1 data and PG1/PG2 ratio – as according to the manufacturing cut-offs these results do not support the CAG status, please explain and discuss.

Author reply to reviewer:

Authors have made following explanation and discussed in the text in Discussion part, lines 367-369 and 402-406.

Application of G-17, PG I, PG II levels and PG I/PG II ratios in gastric cancer epidemiology study have been reported extensively, however, their predicative value in stepwise gastric disease progression in clinical setting have not been very well defined. Previously studies have generated inconsistent results, our results using AUROC analysis (Fig. 2) indicated relatively low predictive value range from 0.529-0.786 for PU, CAG and GC patients, and type I *H. pylori* infection exert important impact on their level as disease progress. The data provide insight to evaluate their application during clinical practice and are helpful to explain the results.

In CAG patients, we also noticed that our PGI level and PG I/PG II ratios are slightly higher when compared with other reports, and as all the CAG patients have histological confirmation, we therefore consider this effect could be patient population- or region-based variations, or might be variations from the degrees of atrophy itself. However future studies are required to explain these discrepancies.

- Language needs editing - Biostatistics review certificate is with inappropriate language

Author reply to reviewer:

The author has modified and uploaded the correct Biostatistics review certificate.

Authors are very grateful to the above three reviewers for their helpful advices, and their time, attention to make the manuscript a much better shape.